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GC-MS ANALYSIS OF MICROORGANISM MARKERS IN PLANTS (REVIEW)

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ABSTRACT *The presence of microorganisms in plants can have a significant impact on their growth, development and general health. Traditional methods for identifying and characterizing these microorganisms can be time consuming and often lack specificity. In recent years, gas chromatography-mass spectrometry (GC-MS) has become a powerful method for the analysis of microorganism markers in plants. This review article presents the principles and applications of GC-MS in the analysis of microorganism markers, indicating its advantages and limitations. In addition, it discusses various case studies in which GC-MS has been successfully used to detect and identify microorganisms in plants, paving the way for improved plant disease control strategies.*

Keywords *microorganism, marker, fatty acids, gas chromatography, analysis.*

Relevance:

Plants are known to contain a wide range of microorganisms, including bacteria, fungi, and viruses, which can have both positive and negative effects on plant health [1-3]. The identification and characterization of these microorganisms is critical to understanding their role in plant-microbial interactions. GC-MS, with its high sensitivity and the ability to obtain detailed chemical information, has become a valuable tool in this field. In this regard, for completeness of information, it is necessary to consider the following sections:

Principles of GC-MS. GC/MS principles include the following [4-8]:

1. **Separation of a mixture of components:** GC-MS is based on the separation of a mixture of analytes into individual components using gas chromatography . This is achieved due to the different affinities of the components for the stationary phase (stationary column) and the mobile phase (mobile gas).

2. **Ionization of the components:** After the separation of the components, they are ionized, which allows them to turn into ions. Various methods are used for this, such as electron ionization (EI), chemical ionization (CI) or electronic shielding (EI).

3. **Mass analysis ionized components:** The ionized components pass through a mass analyzer, which separates the ionized particles by mass and charge. Typically, GC/MS uses mass filters such as a quadrupole or time line device (TOF).

4. **Detection and registration of ions:** Ions are registered by a detector and further processed using a mass spectrometer. This allows you to determine the mass and relative content of each component in the sample.

5. **Component Identification:** To identify components, mass spectrum databases are used, which contain information on the mass spectra of various substances. By comparing the resulting mass spectrum with databases, the structure can be determined and analytes identified.

These principles form the basis of GC-MS analysis and allow high accuracy and sensitivity in the determination of components in various samples.

Microorganism markers in plants. This section discusses the different types of microorganism markers that can be analyzed using GC/MS. It covers volatile organic compounds (VOCs), non-volatile metabolites, fatty acids, and other biomarkers that can serve as indicators of the presence of microbes in plants.

Sample preparation methods in GC-MS.

Sample preparation methods in gas chromatography -mass spectrometry (GC-MS) may vary depending on the type of sample being analyzed and the information required.

The following are some of the main methods for sample preparation in GC/MS:

➢ **Extraction:** This method is used to extract analytes from the sample matrix. For this, various solvents are used, such as acetonitrile , chloroform, diethyl ether, etc. The extraction process can be carried out using ultrasonic treatment or cold irrigation.

➢ **Evaporation:** In this method, the sample is subjected to heat treatment to concentrate the analytes. Heating can be carried out in a water bath, steam bath, vacuum film evaporator or other special devices.

➢ **Direct Injection:** This method involves directly injecting the sample into the GC/MS for analysis. The sample, usually dissolved in a solvent, is injected through the apparatus into the gas chromatograph column .

➢ **Derivatives:** Sometimes analytes can be chemically modified to improve their stability or detectability . Some common derivative methods include derivatization amine o - or carbonyl groups, the conversion of alkanes into gasoline or aromatic compounds, and others.

➢ **Fractionation:** This method is used when the sample contains many analytes with different physicochemical properties. In such cases, the sample may be fractionated based on various filtration, extraction or chromatography techniques.

The choice of the appropriate GC/MS sample preparation method depends on the purpose of the analysis, the type of sample and its matrix, and the capabilities and limitations of the GC/MS setup. It is important to take into account the specific requirements of each assay and tailor sample preparation methods to meet these requirements.

Application of GC-MS in the analysis of microorganisms. This section provides a comprehensive overview of the use of GC-MS in the analysis of microorganism markers in plants. It discusses case studies where GC-MS has been successfully used to detect and identify specific micro-organisms such as fungal pathogens, bacterial endophytes , and viral infections. In addition, it highlights the use of GC-MS to study plant-microbial interactions and the role of micro-organisms in plant diseases.

The importance of GC-MS in the analysis of microbial markers in plants cannot be overestimated. Its ability to provide detailed chemical information about the metabolites of microorganisms provides valuable insight into the complex relationships between plants and microorganisms. Using the power of GC/MS, researchers can unravel the mysteries of plant-microbial interactions, leading to the development of sustainable and effective strategies to protect plant health in the face of microbial challenges.

Microorganisms can leave various markers or signatures that can be used for their detection and identification [9-12]. Here are some commonly studied microorganism markers (Table 1):

Microorganism markers	➢ Volatile organic compounds ➢ Fatty acid ➢ Metabolites ➢ Cell wall components ➢ Nucleic acids ➢ Proteins and enzymes ➢ Biomolecules and metabolic pathways

1. Volatile Organic Compounds (VOC): Microorganisms can produce a wide range of volatile compounds that can serve as markers. These include, among others, alcohols, aldehydes, ketones, esters and sulfur compounds. VOCs are often responsible for characteristic odors associated with microbial growth [13–16].

2. Fatty acids: The fatty acid composition of microorganisms may vary by species and strain. Fatty acid profiles can be used as markers for the identification and classification of microorganisms [17-21].

3. Metabolites: Microorganisms produce specific metabolites during their metabolic activity. These metabolites, such as secondary metabolites, can serve as markers of microbial presence and activity [22–25]. Examples include antibiotics, mycotoxins and pigments.

4. Cell wall components: The composition and structure of cell wall components can vary between microorganisms, providing characteristic markers for their identification [26-29]. For example, peptidoglycan is a major component of bacterial cell walls.

5. Nucleic acids: The genetic material of microorganisms, such as DNA and RNA, can serve as a marker for their detection and identification. Certain gene sequences or regions can be targeted using molecular techniques such as polymerase chain reaction (PCR) for microbial identification [30–34].

6. Proteins and enzymes. Microorganisms express unique proteins and enzymes that can act as markers. These markers can be targeted using immunological methods such as enzyme-linked immunosorbent assay (ELISA) to detect and identify microorganisms [35-38].

7. Biomolecules and metabolic pathways. Microorganisms have different metabolic pathways and produce specific biomolecules . They may include amino acids, sugars, organic acids, and enzymes that can be used as markers for the presence and activity of microbes.

It is important to note that microorganism markers may vary depending on the specific species, strain, and environmental conditions. Researchers often use a combination of markers and analytical methods such as gas chromatography-mass spectrometry (GC-MS) to achieve accurate and reliable identification and characterization of microorganisms in a variety of samples.

Fatty acids are commonly used as markers for the identification and classification of microorganisms. The composition and relative amount of fatty acids can vary among different species and strains of microbes, making them useful for distinguishing microorganisms. Here are some examples of fatty acids that act as microbial markers (Table 2):

Fatty acid markers	➢ Branched-chain fatty acids (BCFAs)
	➢ Monounsaturated fatty acids (MUFAs)

- Polyunsaturated fatty acids (PUFAs)
- Cyclopropane fatty acids (CFA)
- Hydroxy fatty acids
- Saturated fatty acids (SFAs)
- Odd chain fatty acids
- Short chain fatty acids (SCFA)
- Long chain fatty acids (LCAs)
- Omega-3 fatty acids
- Hydroxylated fatty acids
- Polyhydroxyalkanoates (PHA)
- Omega 9 fatty acids
- conjugated fatty acids
- Iso/ anteiso fatty acids
- 3-hydroxy fatty acids
- Mycolic acids
- Gopanoids
- Wax esters
- Sterols
- Fatty acid phospholipids (PLFA)
- Lipopolysaccharides (LPS)
- Essential lipids
- Very long chain fatty acids (VLCFA)
- diacylglycerols

1. Branched Chain Fatty Acids (BCFAs): BCFAs are commonly found in bacterial cell membranes and can be used as markers to identify bacteria. Some examples of BCFAs include o- and anteiso -fatty acids such as iso-C15:0 and iso-C17:0 [13,15,39-44].

2. Monounsaturated fatty acids (MUFAs): MUFAs are fatty acids with one double bond in their hydrocarbon chain. Microorganisms often display specific MUFA patterns that can be used for microbial identification. Examples include oleic acid (C18:1) and palmitoleic acid (C16:1) [13,39,45-47].

3. **Polyunsaturated fatty acids (PUFAs):** PUFAs are fatty acids with several double bonds in their hydrocarbon chain. Although the presence of certain PUFAs in microorganisms is less common than in higher organisms, they may be indicative of certain types of microbes. Examples include linoleic acid (C18:2) and linolenic acid (C18:3) [48-51].

4.Cyclopropane fatty acids (CFAs): Some bacteria produce CFAs, which are fatty acids with one or more cyclopropane rings in their structure. The presence of CFAs can be a characteristic marker for certain bacterial species. Cyclopropane fatty acids such as cyclopropane-C19:0 are commonly used for microbial identification [15,39,49,52-54].

5.Hydroxy fatty acids: Hydroxy fatty acids are fatty acids that contain one or more hydroxyl groups in their structure. These fatty acids are often produced by certain types of bacteria and can serve as markers for their identification [55-58]. Examples include 3-hydroxy fatty acids such as 3-hydroxydecanoic acid (C10:0 3-OH) and 3-hydroxytetradecanoic acid (C14:0 3-OH).

6. Saturated fatty acids (SFA). Saturated fatty acids are fatty acids that do not have double bonds in the hydrocarbon chain. Although SFAs are not as specific as other types of fatty acids, their relative abundance and distribution can provide insight into microbial communities [14,47,49]. Examples of SFAs include stearic acid (C18:0) and palmitic acid (C16:0).

7. Odd-chain fatty acids. Odd chain fatty acids have an odd number of carbon atoms in their structure, such as heptadecanoic acid (C17:0) and nonadecanoic acid (C19:0). These fatty acids can be produced by some microorganisms and can serve as markers for their identification [15,60-64].

8.Short-Chain Fatty Acids (SCFAs): SCFAs typically contain less than six carbon atoms and are produced by microbial fermentation. Examples include acetic acid (C2:0) and propionic acid (C3:0). SCFAs play an important role in microbial ecology and can be used as markers of specific microbial metabolic activity [65–68].

9. Long Chain Fatty Acids (LCFAs): LCFAs are fatty acids with more than 14 carbon atoms. They can be found in a variety of microorganisms, and their relative abundance can provide insight into the composition of a microbial community. Examples include behenic acid (C22:0) and arachidic acid (C20:0) [13,14,64].

10. Omega-3 fatty acids: Omega-3 fatty acids are polyunsaturated fatty acids with a double bond at the third carbon atom from the omega end of the carbon chain. They are commonly found in some microorganisms, especially marine bacteria and algae. Examples of omega-3 fatty acids include eicosapentaenoic acid (EPA, C20:5ω3) and docosahexaenoic acid (DHA, C22:6ω3). The presence of omega-3 fatty acids can serve as a marker for certain types or groups of microbes [69-74].

11. Hydroxylated fatty acids: Hydroxylated fatty acids are fatty acids that contain one or more hydroxyl groups attached to the carbon chain. These fatty acids are often produced by some bacteria as secondary metabolites and may act as signaling molecules or play a role in host-microbe interactions. Examples include 10-hydroxy-2-decanoic acid (C10:0 10-OH) produced by some Gram-negative bacteria [13,75-78].

12.Polyhydroxyalkanoates (PHA): Polyhydroxyalkanoates are a class of microbial polyesters synthesized by some bacteria as intracellular storage compounds. PHAs are composed of various hydroxyalkanoic acids and can serve as markers for the presence of bacteria capable of producing these polymers [79-82].

13. Omega-9 fatty acids: Omega-9 fatty acids are monounsaturated fatty acids with a double bond at the ninth carbon atom from the omega end of the carbon chain. Although omega-9 fatty acids are also found in higher organisms, they can be used as markers for certain microbial species or strains [83-86].

14. Conjugated fatty acids: Conjugated fatty acids are characterized by the presence of several conjugated double bonds. These fatty acids are produced by some bacteria and can serve as markers for certain microbial groups. Examples include conjugated linoleic acid (CLA) produced by lactic acid bacteria [87,88].

15.Iso/ anteiso fatty acids: Iso and anteiso fatty acids are branched chain fatty acids that contain a methyl group attached to a carbon atom adjacent to a carboxyl group. These fatty acids are commonly found in bacteria and can serve as markers for specific bacterial groups or taxonomic identification. Examples include iso-C15:0 and iso-C17:0 [89-90].

16.3-hydroxy fatty acids: 3-hydroxy fatty acids are fatty acids containing a hydroxyl group at the third carbon atom. These fatty acids are produced by some bacteria as components of complex lipids and can act as markers for certain species or groups of microbes. Examples include 3-hydroxydecanoic acid (C10:0 3-OH) and 3-hydroxytetradecanoic acid (C14:0 3-OH) [49,75,91-93].

17. Mycolic acids: Mycolic acids are long chain fatty acids with a unique structure that are found in the cell walls of some bacteria, especially mycobacteria. These fatty acids contribute to the characteristic waxy appearance of the cell wall and can serve as markers for the presence of mycobacteria [94,95].

18. Gopanoids: hopanoids are bacterial lipids that are structurally similar to sterols that are found in eukaryotes. These lipids are widely distributed in bacteria and can act as biomarkers for bacterial populations. Hopanoids have been used as markers for the presence of certain groups of bacteria, such as cyanobacteria and some proteobacteria [96-99].

19. Wax esters: Wax esters are ester compounds formed by the esterification of fatty acids with long chain alcohols. Some bacteria and fungi can produce wax esters, and their presence can serve as a marker for the activity of certain micro-organisms or metabolic processes. Wax esters have been studied in various environments, including marine systems and soil microbial communities [13,100-104].

20. Sterols: Although sterols are more commonly associated with eukaryotes, some bacteria, such as some Mycoplasma species , are able to synthesize sterol-like compounds called bacteriohopan polyols (BHPs). These compounds can serve as markers for the presence of certain groups of bacteria and have been used in paleoecological studies as biomarkers of microbial activity [15,105-108].

21.Phospholipids of fatty acids (PLFA): Phospholipids of fatty acids are the fatty acid components of phospholipids, which are the main constituents of the cell membranes of microorganisms. PLFA analysis can provide insight into microbial biomass, community structure, and functional diversity. Various PLFAs can be used as markers for certain microbial groups or metabolic activity [39,46,109-115].

22. Lipopolysaccharides (LPS): Lipopolysaccharides are complex molecules found in the outer membrane of Gram-negative bacteria. They consist of a lipid component (lipid A) and a polysaccharide component. The fatty acid composition of lipid A can vary between bacterial species and strains, and LPS analysis can provide markers for the presence and identification of specific Gram-negative bacteria [116-119].

23.Ether lipids: While most microorganisms contain ester-linked fatty acids, some archaea, such as methanogens , produce ester lipids in their cell membranes. Ether lipids have unique structural characteristics and can serve as markers for the presence of specific archaeal groups [51,120].

24. Very Long Chain Fatty Acids (VLCFA): Very long chain fatty acids generally refer to fatty acids with a carbon chain length of 20 or more carbon atoms. These fatty acids are found in various microorganisms and can serve as markers for specific microbial groups or metabolic activity. VLCFAs have been studied in the context of soil microbial communities and their potential role in nutrient cycling [64,69,121-124].

25. Diacylglycerols: Diacylglycerols are lipid molecules composed of two fatty acid chains attached to a glycerol backbone. They are important intermediates in lipid metabolism and membrane biosynthesis [13,121,125-127].

Advantages of GC-MS [1-4] :

- **High Sensitivity:** GC-MS is one of the most sensitive methods for analyzing organic compounds. It allows the detection and determination of substances at very low concentrations, which is especially important for medical, food and environmental analysis.
- **High specificity:** GC-MS provides excellent recognition specificity and a unique ability to determine the molecular structure of the analyzed compounds. This allows you to get more accurate and reliable results.
- **Ability to analyze a wide range of compounds:** GC-MS can be used to analyze various classes of organic compounds, including volatile and thermostable substances, aromatic and heterocyclic compounds, polymers, and even living tissues.
- **Fast and automated:** GC-MS is a fast and relatively easy-to-use method of analysis. Modern instruments have the ability to automate sample insertion and data processing, minimizing human error and increasing productivity.

Limitations of GC/MS [5-9] :

- **High equipment cost:** Acquisition and maintenance of GC/MS equipment is a significant financial investment, especially for high performance and multi-channel systems.

➢ **Difficulty in interpreting data:** Interpreting mass spectral data can be complex and requires specific knowledge and skills. In some cases, additional analysis or database comparison may be required to fully identify compounds.

➢ **Insufficient Resolution:** Some compounds may experience peak overlapping problems, especially when analyzing complex mixtures. This can complicate identification and quantification.

➢ **Possibility of analyte degradation:** High temperatures, use of catalytic surfaces, and other operating conditions in GC/MS can degrade some compounds. This may lead to false results or the inability to analyze certain substances.

Future prospects for GC-MS and conclusions.

The future prospects of gas chromatography -mass spectrometry (GC-MS) promise to be very promising in many scientific and industrial fields. Here are a few key takeaways about the future of this technology:

➢ **Instrumentation Developments:** Significant developments in GC/MS equipment are expected over time, including improved detector sensitivity, increased resolution, and increased analysis speed. This will allow researchers to obtain more accurate and reliable analysis results.

➢ **Expanding Applications:** GC/MS has already been successfully applied in a variety of applications including food, environmental, pharmaceutical, oil refining, and more. In the future, GC-MS is expected to find new applications such as forensics, medicine, and biological research.

➢ **Methodological evolution:** With the development of GC/MS, new and improved methods of analysis are expected. For example, multivariate GC/MS allows you to get more detailed information about the composition of samples, as well as integrate data from various sources for a more complete analysis.

➢ **Application of artificial intelligence and machine learning:** In the future, the use of artificial intelligence and machine learning methods in the analysis of GC-MS data is expected to increase. This will automate and speed up the analysis process, as well as facilitate the interpretation of the results.

All in all, the future prospects of GC/MS promise to be very attractive and useful for research and industry. This technology will continue to evolve, opening up new possibilities for analysis and allowing a more complete understanding of complex chemical and biological systems.

The choice and identification of specific fatty acid markers depends on the focus of the study and the target microorganisms. The use of fatty acids as markers in microbial analysis provides valuable insight into microbial community structure, metabolic activity, and ecological functions. Advanced analytical techniques such as chromatography, mass spectrometry, and lipidomics have contributed greatly to the identification and characterization of fatty acid markers in microorganisms.

Overall, the analysis of fatty acids as markers in microorganisms provides valuable information for microbial identification, analysis of community structure, and understanding of the functional roles of microorganisms in various environments. Techniques such as gas chromatography-mass spectrometry (GC-MS) are commonly used to analyze fatty acids, allowing researchers to gain insight into microbial communities and their role in plant-microbial interactions, environmental processes, and human health.

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